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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/026,914	12/27/2001	Birgit Linhart	0273-0006	6890

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EXAMINER

HINES, JANA A

ART UNIT

PAPER NUMBER

1645

DATE MAILED: 01/13/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 10/026,914	Applicant(s) LINHART ET AL.5	
	Examiner Ja-Na Hines	Art Unit 1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
 - If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
 - If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
 - Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 04 October 2004.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-7,9,13-15 and 20-26 is/are pending in the application.
- 4a) Of the above claim(s) 7,9,22-25 is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-6,13-15,20,21 and 26 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
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Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Amendment Entry

1. The amendment filed October 4, 2004 has been entered. Claims 1 and 26 have been currently amended. Claims 8, 10-12 and 16-19 have been cancelled. Claims 7,9, and 22-25 have been withdrawn. Claims 1-6, 13-15, 20-21 and 26 are under consideration in this office action.
2. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

Withdrawal of Rejections

3. The following rejection has been withdrawn in view of applicants' amendments and arguments:

The rejection of claims 1-3 and 13-14 under 35 U.S.C. 102(b) as being anticipated by Vrtala et al., (1996. J. Allergy Clin. Immun. Vol. 97(3): 781-787).

Response to Arguments

4. The rejection of claims 13-14 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement is maintained. The rejection was on the grounds that the specification and claims lack sufficient written description of the polynucleotide encoding the hybrid polypeptide. Applicants' assert that the polynucleotide and polypeptide sequences for the timothy grass pollen allergens were available in the art. However the claims are not just drawn to the entire polynucleotide and polypeptide sequences. Rather the claims encompass fragments thereof wherein each fragment consists of at

least eight consecutive amino acids from the respective allergenic proteins. There is no description of the fragments of nucleic acids that must encode the hybrid polypeptide. The instant specification does not provide for a method for preparing a hybrid polypeptide comprising fragments of polynucleotide. The specification does not provide a teaching of the fragmented structure, showing that nucleic and amino acid fragments were isolated at the time the invention was made, thus there is no teaching of a preparation method.

Applicants' urged that one of ordinary skill in the art armed with the instant specification, would understand the sequences used in the present invention. However, the standard is not that one would understand the sequences used in the present invention. To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. See, e.g., *Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed. Cir. 2003); *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116. Applicants' have not even pointed to support for the fragments thereof wherein each fragment consists of at least eight consecutive amino acids from the respective allergenic proteins. Thus no preparation method has been disclosed. Rather applicants' have disclosed the entire sequences but have failed to disclose a method for preparing a hybrid polypeptide comprising fragments consisting of at least eight consecutive amino acids from the respective allergenic proteins.

Possession may be shown in a variety of ways including description of an actual reduction to practice, or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing distinguishing

identifying characteristics sufficient to show that the applicant was in possession of the claimed invention. See, e.g., *Pfaff v. Wells Elecs., Inc.*, 525 U.S. 55, 68, 119 S.Ct. 304, 312, 48 USPQ2d 1641, 1647 (1998); *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406; *Amgen, Inc. v. Chugai Pharmaceutical*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991). Applicants' have failed to show that they were in possession of such. Moreover, it is noted that functional limitations alone are not sufficient to satisfy the written description requirement.

There is no conception of a method for preparing a hybrid polypeptide comprising fragments thereof as claimed at the time of filing. Furthermore, applicants have not taught what fragments will encode polynucleotides which are capable of encoding the polypeptide. There is no teaching of a representative fragment polynucleotide encoding a fragment of a polypeptide. In view of applicants' failure to explain the essential details the rejection is maintained.

Thus, in the absence of sequence information as claimed applicants arguments, declaration and amendments are not persuasive.

5. The written description rejection of claims 1-6, 13-15, 20-21 and 26 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement is maintained. The claim(s) contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

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The claims are drawn to a hybrid polypeptide comprising at least two different plant allergenic proteins or fragments thereof, wherein the plant allergenic proteins are selected from the group consisting of Phl p1, Phl p2, Phl p5 and Phl p6 and wherein each fragment consists of at least eight consecutive amino acids of the respective allergenic protein and said hybrid polypeptide induces an antibody response.

Applicants assert that by amending the claims to recite the function of inducing an antibody response the rejection should be withdrawn. However, the specification fails to teach how to define fragments thereof with respect to which eight consecutive amino acids must be comprised therein to acquire the appropriate fragments. Neither the claims nor the specification teach how to obtain such fragments thereof. There is no guidance as to what amino acids may or may not be included without causing a detrimental effect to the fragments thereof as claimed. The claims broadly recite fragments thereof, therefore any fragment is being claimed, and no specific location requirement for particular amino acids is recited. Thus, the resulting fragments thereof could result in a functional fragment not taught and enabled by the specification. There is no written description of which eight amino acids must be comprised in the claimed hybrid polypeptide. Applicants' amendment does not overcome this lack of written description.

Applicants assert that because they have provided a generic definition of fragments at pages 2-3 of the instant specification and because the entire sequence is known one of skill in the art would understand that they were in

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possession. However, this argument is not persuasive. With the exception of specifically recited sequences the skilled artisan cannot envision the detailed structure of the fragments thereof, thus conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. An adequate description requires more than a mere statement that it is part of the invention and a reference to a potential method of isolating it. The nucleic acid and amino acid fragment sequences themselves are required. See *Fiers v. Revel*, 25 USPQ 2d 1601 at 1606 (CAFC 1993) and *Amgen Inc. V. Chugai Pharmaceutical Co. Lts.*, 18 USPQ2d 1016.

A lack of adequate written description issue arises if the knowledge and level of skill in the art would not permit one skilled in the art to immediately envisage the product claimed from the disclosed process as is the case here. Applicants' specification point out fragments can exist, however there is no disclosure of even one representative fragment. Thus one of skill in the art could not immediately envision the claimed fragments. See, e.g., *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1571, 39 USPQ2d 1895, 1905 (Fed. Cir. 1996) (a "laundry list" disclosure of every possible moiety does not constitute a written description of every species in a genus because it would not "reasonably lead" those skilled in the art to any particular species); *In re Ruschig*, 379 F.2d 990, 995, 154 USPQ 118, 123 (CCPA 1967) ("If n-propylamine had been used in making the compound instead of n-butylamine, the compound of claim 13 would have resulted. Appellants submit to us, as they did to the board, an imaginary

specific example patterned on specific example 6 by which the above butyl compound is made so that we can see what a simple change would have resulted in a specific supporting disclosure being present in the present specification. The trouble is that there is no such disclosure, easy though it is to imagine it.") (emphasis in original); *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1328, 56 USPQ2d 1481, 1487 (Fed. Cir. 2000) ("the specification does not clearly disclose to the skilled artisan that the inventors ... considered the ratio... to be part of their invention There is therefore no force to Purdue's argument that the written description requirement was satisfied because the disclosure revealed a broad invention from which the [later-filed] claims carved out a patentable portion". Similarly, it appears that the instant case sets forth undisclosed fragments and asserts that these undisclosed fragments have some functional limitations. However, there is no actual disclosure of the claimed fragments. Moreover, the functional limitations do not constitute a written description of every species in a genus because it would not "reasonably lead" those skilled in the art to any particular species.

Applicants assert that they have conveyed with clarity to those skilled in the art that they were possession of the invention. However, at best applicants have shown that they were in possession of the entire sequence of timothy grass allergens, but applicants have not shown that they were in possession of fragments capable of inducing an antibody response. There is no disclosure of a highly conserved and immunogenic region in the plant allergen. Therefore, the specification lacks adequate support for the claims. Furthermore, *In The*

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Reagents of the University of California v. Eli Lilly (43 USPQ2d 1398-1412), the court held that a generic statement which defines a genus of amino acids by only their functional activity, i.e., inducing an antibody response does not provide an adequate description of the genus. The court indicated that while Applicants are not required to disclose every species encompassed by a genus, the description of a genus is achieved by the recitation of a representative number of DNA molecules, usually defined by a nucleotide sequence, falling within the scope of the claimed genus. At section B(1), the court states that "An adequate written description of a DNA...requires a precise definition, such as by structure, formula, chemical name, or physical properties', not a mere wish or plan for obtaining the claimed chemical invention".

Currently the instant claims lack an adequate description of the fragments thereof, thus the descriptions are insufficient to support the claims as provided by the Interim Written Description Guidelines published in the June 15, 1998 Federal Register at Volume 63, Number 114, pages 32639-32645. Therefore, the full breadth of the claims fails to meet the written description provision of 35 USC 112, first paragraph and the rejection are maintained.

New Grounds of Rejection

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any

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person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims 1-6, 13-15, 20-21 and 26 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection

Neither the specification nor originally presented claims provides support for a hybrid polypeptide comprising at least two different plant allergenic proteins or fragments thereof, wherein the plant allergenic proteins are selected from the group consisting of Phl p1, Phl p2, Phl p5 and Phl p6 and wherein each fragment consists of at least eight consecutive amino acids of the respective allergenic protein and said hybrid polypeptide induces an antibody response.

Applicant did not point to support in the specification for a hybrid polypeptide comprising fragments thereof wherein each fragment consists of at least eight consecutive amino acids of the respective allergenic protein and said hybrid polypeptide induces an antibody response. Moreover, applicant failed to specifically point to the identity or provide structural characteristics of fragments thereof wherein each fragment consists of at least eight consecutive amino acids of the respective allergenic protein and said hybrid polypeptide induces an antibody response. Thus, there appears to be no teaching of fragments thereof wherein each fragment consists of at least eight consecutive amino acids of the

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respective allergenic protein and said hybrid polypeptide induces an antibody response.

Applicant has pointed to pages 2-3, 8 and 16-17 of the instant specification and claims for support of the amendment which are drawn to the fragments thereof wherein each fragment consists of at least eight consecutive amino acids of the respective allergenic protein and said hybrid polypeptide induces an antibody response, however it appears that the entire specification appears to fail to recite support for the new fragments. There is no teaching of any isolated fragments. There is no teaching of any fragments being comprised within a hybrid polypeptide. There is no teaching of a hybrid polypeptide comprising such fragments as inducing an antibody response in any host. Therefore, it appears that there is no support in the specification. Therefore, applicants must specifically point to page and line number support for the identity of fragments thereof wherein each fragment consists of at least eight consecutive amino acids of the respective allergenic protein and said hybrid polypeptide induces an antibody response as recited by the newly added amendments. Therefore, the new claims incorporate new matter and are accordingly rejected.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to

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be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

7. Claims 1-3 and 13-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ball et al., (WO 95/34578) in view of Vrtala et al., (1996. J. Allergy Clin. Immun. Vol. 97(3): 781-787).

The amended claims are drawn to a hybrid polypeptide comprising at least two different plant allergenic proteins or fragments thereof, wherein the plant allergenic proteins are selected from the group consisting of Phl p1, Phl p2, Phl p5 and Phl p6 and wherein each fragment consists of at least eight consecutive amino acids of the respective allergenic protein and said hybrid polypeptide induces an antibody response.

Ball et al., teach the major grass pollen allergen Phl p1. The recombinant DNA molecule may contain a nucleotide sequence which codes for a polypeptide which would induce an antibody response (page 3 lines 20-25). The invention teaches a recombinant or synthetic protein or polypeptide comprising as an essential part Phl p1 (page 3 lines 33-35). The protein or polypeptide may be fused to an additional polypeptide, such as any other polypeptide that can be expressed as a fusion protein in prokaryotic or eukaryotic cells (page 4 lines 1-4). The invention also includes a recombinant DNA expression vector or cloning system (page 3 lines 26-30). Ball et al., while teaching that the Phl p1 can be part of a hybrid or fusion polypeptide does not specifically recite using another plant allergenic protein within the hybrid polypeptide.

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Vrtala et al., teach grass pollen allergens belong to the potent elicitors of type I allergy (abstract). Vrtala et al., teach that DNA coding for three major timothy grass pollen allergens representing group I (Phl p1), group II (Phl p 2) and group V (Phl p 5) was known (page 781). There is no relevant immunologic similarity between Phl p 2 and Phl p 1 (page 781). The methods section teaches the construction of the expression plasmids for Phl p 1, Phl p 2 and Phl p 5 (page 782). cDNA clones were transcribed by polymerase chain reaction to DNA fragments coding for the mature allergens (page 782). Phl p 1 and Phl p 2, both of which contained ATG start codon in front of the coding region of the mature protein and genes were then inserted as fragments (page 782). The plasmids were transfected into *E.coli* host cells. The expression of the recombinant allergens in *E.coli* was also taught wherein cells were cultured, expressed, purified and thereby recovered (page 782).

Therefore it would have been prima facie obvious at the time of applicants' invention to modify the hybrid polypeptide as taught by Ball et al., to include a different plant allergen as taught by Vrtala et al., since Ball et al., already teach the need to have a hybrid or fusion polypeptide. Ball et al., teach that plant allergenic proteins such as Phl p1 are amenable to being comprised within fusion proteins and/or hybrid polypeptides and can be fused to any other polypeptide that can be expressed as a fusion protein in prokaryotic or eukaryotic cells, while Vrtala et al., teach polypeptides that can be expressed in prokaryotic or eukaryotic cells, thus no more than routine skill would have been required to create a hybrid polypeptide comprising at least two different plant allergens. Thus,

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there is a reasonable expectation of success in using the Phl pl of Ball et al., and any other polypeptide such as the ones taught by Vrtala et al., when the prior art teaches that all of these plant allergens can be expressed as a fusion protein in prokaryotic or eukaryotic cells.

Conclusion

8. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire **THREE MONTHS** from the mailing date of this action. In the event a first reply is filed within **TWO MONTHS** of the mailing date of this final action and the advisory action is not mailed until after the end of the **THREE-MONTH** shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than **SIX MONTHS** from the date of this final action.


9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ja-Na Hines whose telephone number is 571-

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272-0859. The examiner can normally be reached on Monday-Thursday and alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Lynette Smith can be reached on 571-272-0864. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Ja-Na Hines, 
January 4, 2005


LYNETTE R. F. SMITH
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Notice of References Cited	Application/Control No. 10/026,914	Applicant(s)/Patent Under Reexamination LINHART ET AL.5	
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U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
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	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N	WO 95/34578	12-1995	World	Ball et al.	C07K 14/415
	O					
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	Q					
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	S					
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: C07K 14/415, C12N 15/29, A61K 39/36, C07K 14/735	A1	(11) International Publication Number: WO 95/34578 (43) International Publication Date: 21 December 1995 (21.12.95)
(21) International Application Number: PCT/SE95/00724 (22) International Filing Date: 14 June 1995 (14.06.95) (30) Priority Data: 9402089-8 14 June 1994 (14.06.94) SE (71) Applicant (for all designated States except US): PHARMACIA AB [SE/SE]; S-171 97 Stockholm (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): BALL, Tanja [AT/AT]; Myrthengasse 5/4, A-1070 Wien (AT). VRTALA, Susanne [AT/AT]; Schenkendorfgasse 14-16/1/9, A-1210 Wien (AT). SPERR, Wolfgang [AT/AT]; Iglaseegasse 9, A-1190 Wien (AT). VALENT, Peter [AT/AT]; Schulgasse 7/18, A- 1170 Wien (AT). SUSANI, Markus [AT/AT]; Laufenstrasse 30, A-5020 Salzburg (AT). KRAFT, Dietrich [AT/AT]; Rebenweg 1/18/1, A-1170 Wien (AT). VALENTA, Rudolf [AT/AT]; Beethovenstrasse 18, A-2604 Theresienfeld (AT). LAFFER, Sylvia [AT/AT]; Krichbaumgasse 42/4, A-1120 Wien (AT). (74) Agents: BERGANDER, Håkan et al.; Pharmacia AB, Patent Dept., S-751 82 Uppsala (SE).		(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
(54) Title: RECOMBINANT ALLERGEN, FRAGMENTS THEREOF, CORRESPONDING RECOMBINANT DNA MOLECULES, VECTORS AND HOSTS CONTAINING THE DNA MOLECULES, DIAGNOSTIC AND THERAPEUTIC USES OF SAID ALLERGENS AND FRAGMENTS		
(57) Abstract <p>A recombinant DNA molecule comprising a nucleotide sequence (I) which codes for a polypeptide displaying the antigenicity of one, two or more of the Phl p I epitope clones (28, 34, 41, 42, 43, 50, 52, 64, 80, 85, 86, 95, 97, 98, 103, 108, 109, 113, 114) with the amino acid sequences defined in figure 2 and preferably being derived from grasses or monocotyledonic plants, or a nucleotide sequence (II) which hybridizes with such a nucleotide sequence (I) under conditions of high stringency. Polypeptides displaying the antigenicity of one, two or more of the Phl p I epitope clones (28, 34, 41, 42, 43, 50, 52, 64, 80, 85, 86, 95, 97, 98, 103, 108, 109, 113, 114) with the amino acid sequences defined in figure 2. Recombinant expression vectors containing the recombinant molecule and host cells transformed with the vector. Diagnostic methods based on utilizing the polypeptides in immunoassays for humoral antibodies and cellular reactions.</p>		

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**RECOMBINANT ALLERGEN, FRAGMENTS THEREOF, CORRESPONDING RECOMBINANT
DNA MOLECULES, VECTORS AND HOSTS CONTAINING THE DNA MOLECULES,
DIAGNOSTIC AND THERAPEUTIC USES OF SAID ALLERGENS AND FRAGMENTS.**

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The present invention relates to the title aspects of the major grass pollen allergen Phl p I and IgE-binding epitopes present in this allergen and corresponding haptens. The invention also relates to fragments, including IgE-binding haptens, from other
10 grass and monocotyledonic plant allergens containing the IgE binding epitopes of Phl p I. The invention is primarily concerned with epitopes that normally are found in one or more group I allergens.

15 **Background to the invention**

Up to 20 % of the population in industrialized countries suffer from Type I allergic symptoms (rhinitis, conjunctivitis, asthma bronchiale) (Myamoto et al., 1992). The crosslinking of IgE which is bound to mast cells and basophils via the high affinity
20 receptor FcεRI is the key event leading to release of biological mediators such as histamine (Segal et al., 1977). The crosslinking event by allergens represents, therefore, a potential target for therapy of Type I allergy. Such therapeutical approaches could either use portions of the IgE-
25 molecule or other ligands, to interfere with the binding of IgE to the high affinity Fcε-receptor, or reagents to block the subsequent signal transduction cascade thus preventing the degranulation of mast cells and basophils (Dreskin et al., 1988). An additional possibility for specific therapy would be to use
30 haptens derived from complete allergens which by binding to IgE monovalently could block the crosslinking of IgE (Valenta et al., 1993a). IgE-haptens could also be used to modulate the immune response or to induce tolerance by immunotherapy with a minimum of anaphylactic side effects. Haptens can be obtained from
35 complete allergens by proteolytic digestion. However, this often results in a mixture of fragments and enzymes that are difficult

to characterize. Synthesis of peptides based on the amino acid sequence of the allergens, is an alternative approach. Recently a number of cDNAs coding for important allergens (Scheiner et al., 1992) were isolated which can be used to determine IgE-epitopes by molecular biological techniques.

Grass pollen allergy is spread world wide and according to the prevalence of grass pollen allergy it can be expected that 75 % of all allergic patients suffer from grass pollen allergy (Freidhoff et al., 1986).

Among the grass pollen allergic patients more than 90 % display IgE-reactivity with group I allergens (Freidhoff et al., 1986; Valenta et al., 1992).

The full amino acid sequences and nucleotide sequences of the major grass pollen allergens have been known for some time (timothy grass Phl p I (Laffer et al., 1993), rye grass (Lolium perenne) Lol p I (Perez et al., 1990; Griffith et al., 1991; University of Melbourne WO-A-9203550; Brunet C et al., International Symposium on Molecular Biology of Allergens and the Atopic Response, Quebec City, Canada, February 18-22, 1995; Lamontagne P et al., International Symposium on Molecular Biology of Allergens and the Atopic Response, Quebec City, Canada, February 18-22, 1995), and from rye from timothy grass (Phleum pratense) Sec c I (Laffer et al., unpublished data).

During the priority year the determination of clones 80, 97 and 98 as carriers for a group I conserved IgE binding epitope has been described (Ball et al., 1994a, b, c; Laffer et al., 1994;).

Definition

The term IgE-hapten identifies short allergen fragments on which only one IgE-antibody with a given specificity is allowed to bind. A real IgE-binding hapten will give no histamine release because it contains the binding site for exclusively one IgE antibody. The term epitope in the context of the present invention refers to an IgE-epitope if not otherwise specified. An epitope may be located on either an IgE-hapten or a longer

polypeptide comprising several IgE-binding sites/epitopes. The term IgE preferentially refers to human IgE.

Objectives of the invention

- 5 The objectives of the invention are to provide simple, better and more reliable in vitro and in vivo tests for grass pollen allergy as well as improved therapeutic methods for this disease.

The invention

- 10 A first aspect of the invention is a recombinant DNA molecule comprising a nucleotide sequence (I) which codes for a polypeptide displaying the antigenicity of at least one of the Phl p I epitope clones 28, 34, 41, 42, 43, 50, 52, 64, 80, 85, 86, 95, 97, 98, 103, 108, 109, 113, 114 with the amino acid
15 sequences defined in figure 2 and preferably being derived from grasses or monocotyledonic plants, or a nucleotide sequence (II) which hybridizes with such a nucleotide sequence (I) under conditions of high stringency. The recombinant DNA molecule comprises also degenerate variants of these nucleotide sequences.
20 The recombinant DNA molecule may also contain a nucleotide sequence which codes for a polypeptide having antigenic crossreactivity and a high degree of homology, preferably > 50 % such as > 60 % or > 75 %, with Phl p I epitopes from grasses or other monocotyledonic plants, preferably those defined by the
25 amino acid sequences given in figure 2.

A second aspect of the invention is a recombinant DNA expression vector or cloning system comprising an expression control sequence operatively linked to any of the recombinant molecules defined above.

- 30 A third aspect of the invention is a host cell containing a recombinant molecule or vector according to the first or second aspect, respectively.

- A fourth aspect of the invention is a recombinant or synthetic protein or polypeptide displaying the antigenicity of a Phl p I
35 epitope, in particular comprising as an essential part a Phl p I epitope of at least one of the sequences set out in figure 2. The

protein or polypeptide may be fused to an additional polypeptide, such as β -galactosidase, GST or lambda cII protein or any other polypeptide that can be expressed as a fusion protein in prokaryotic or eukaryotic cells.

5 In the inventive poly/oligonucleotides and proteins/polypeptides, at least one of the sequences defined in figure 2 constitutes an essential part. For the poly-/oligonucleotides this means that each of them should not be longer than half of the DNA sequence coding for the full length Phl p I allergen and
10 preferably containing a nucleotide sequence coding for at least one Phl p I epitope, such as being present in the Phl p I fragments specified in figure 2. The inventive oligo/poly-nucleotides chains are often shorter than 25 % of the DNA coding for the full length Phl p I allergen.

15 For the inventive proteins and polypeptides "essential part" means that each of them should not be longer than half of the full length Phl p I allergen and preferably also contain at least one Phl p I epitope, such as one or more of the epitopes defined by the fragments of full length Phl p I allergen specified in
20 figure 2. The inventive proteins and polypeptides are often shorter than 25 % of the full length Phl p I allergen.

By the expression "a polypeptide displaying the antigenicity of at least one of the clones 28, 34, 41, 42, 43, 50, 52, 64, 80, 85, 86, 95, 97, 98, 103, 108, 109, 113, 114" is meant any peptide
25 portion displaying at least one epitope defined by these clones and being recognizable immunologically. It can be envisaged that polypeptides exhibiting Phl p I epitopes may be derivatized to carry analytically detectable groups or water-soluble or water-insoluble solid phases suitable for immunoassays of antibodies directed against them, e.g. IgA, IgD, IgE, IgM or IgG antibodies.
30 In aspects of the invention relating to in vitro diagnostics (see below) the inventive peptides may be a) linked to a water-insoluble phase by physical adsorption or a covalent bond, or b) conjugated covalently to an analytically detectable group
35 (label).

The fifth aspect the invention is an in vitro method for diagnosing allergy to plant proteins by determining humoral antibodies directed towards the plant proteins. The allergies concerned are mostly against grass pollen. The relevant antibodies are mostly of the IgE class but IgG antibodies may also give information about the allergy. In general this method comprises contacting a body fluid sample derived from a patient with an inventive polypeptide. The amounts and conditions are selected so that an immune complex between the polypeptide and antibodies in the sample are formed in an amount that is a function of the amount of antibodies in the sample. The immune complex is then measured in a per se known manner.

More specifically a preferred method of the fifth aspect comprises contacting a body fluid sample containing the Igs concerned, e.g. IgG or IgE, with a polypeptide according to the invention and an anti-IgE antibody so as to form the immune complex containing peptide:IgE:anti-IgE. Normally either the peptide or the anti-IgE is linked to a solid phase that is insoluble or insolubilizable in the assay media so that the complex can be separated from the assay media. The determination step in these variants may be performed by use of an analytically detectable group (label) that either is covalently linked to the anti-IgE antibody (in case the peptide is linked to the solid phase) or to the peptide (in case the anti-IgE antibody is linked to the solid phase). In case IgG antibodies are to be determined anti-IgG replaces anti-IgE.

A sixth aspect of the invention is a method employing measuring, preferably in vitro, the cellular reaction against a Phl p I epitope. The method comprises using a recombinant or synthetic polypeptide as defined for the fourth aspect to stimulate the cellular reaction. Cellular reactions to be measured are histamine release and T cell proliferation (by ^3H thymidine uptake).

The samples used in the above-mentioned methods are often derived from blood such as whole blood, serum and plasma,

although also other body fluids containing Igs may be used (tears etc).

Commonly accepted solid phase forms useful for immunoassays are walls of microtitre wells, spheres, rods, sheets, strips, pads etc. The solid phase may be porous or non-porous. The material in the solid phase may be a polymer selected among polysaccharides and their derivatives, for instance dextran, pullulan, agarose, cellulose etc, or synthetic polymers, preferably vinyl polymers, such as polyacrylamides, polyacrylates, polystyrene, polyvinyl alcohol etc. The polymers in question are often cross-linked, particularly in case the base polymer as such is water-soluble. Examples of analytically detectable groups are isotopes, enzymes, enzyme substrates, fluorophors, haptens, biotin etc.

A seventh aspect of the invention is a method for the treatment of a mammal, such as a human, which has a pollen allergy by administering a therapeutically effective amount of a recombinant or synthetic polypeptide as defined above. Illustrative examples of the therapeutic aspect of the invention are: a) passive therapy of effector organ (nose, conjunctiva, and lung) to prevent mediator release upon subsequent exposition to the complete allergen, and b) use of the peptides as safe tools for active immunotherapy because a single IgE epitope as such do not release mediators so that high doses can be applied. See further in the Discussion part below. The administration routes will be as commonly applied for current hyposensitization. The doses are likely to be in the $\mu\text{g/ml}$ -range per kg body weight, i.e. 10-100 μg per kg body weight.

EXPERIMENTAL PROCEDURES

A detailed description referring to the procedure for determining one IgE binding epitope encoded by the clones 80, 97 and 98. During the priority year the same method resulted in that further IgE binding epitopes were deduced (clones 28, 34, 41, 42, 43, 50, 52, 64, 85, 86, 95, 103, 108, 109, 113, 114). See Figure 2 for their specific sequences and positions within the Phl p I allergen.

Construction of an epitope cDNA library from the randomly fragmented Phl p I cDNA.

The cDNA fragment coding for Phl p I (Valenta et al., 1992; Laffer et al., 1993) was excised from plasmid pUC 18 and purified by preparative agarose gel electrophoresis (Sambrook et al., 1989; Ausubel et al., 1990). The cDNA was then randomly digested with DNase I (Sambrook et al., 1989; Ausubel et al., 1990) and fragments shorter than 400 bp was isolated by preparative agarose gel electrophoresis. The cDNA fragments were then end repaired with T4 polymerase (Boehringer Mannheim, Germany), linked with 5' phosphorylated 8-mer Eco R I linkers (Schmidheini, Windisch, Switzerland). After Eco R I digestion, linkers were removed using a nick column (Pharmacia Biotech AB, Uppsala, Sweden) and the inserts were ligated into dephosphorylated lambda gt11 arms (Pharmacia Biotech AB, Uppsala, Sweden). The phage DNA was then in vitro packaged using in vitro packaging extracts (Amersham, Buckinghamshire, U.K.). In the recombinant phage particles, the DNA inserts become fused to the gene for β -galactosidase.

20

IgE-immunoscreening and analysis of epitope clones.

100,000 phages of the Phl p I library were used to infect E. coli Y1090 at a density of 5,000 phages per plate (140 mm diameter). The synthesis of recombinant proteins was induced by overlaying the plates with nitrocellulose filters (Schleicher & Schuell, Dassel, Germany) soaked in 10 mM IPTG (Huynh et al., 1985). 114 Phl p I epitope clones were isolated using serum IgE from a Phl p I allergic patient and ^{125}I labeled rabbit anti-human IgE (Pharmacia Diagnostics, Uppsala, Sweden) as described (Breiteneder et al., 1989; Valenta et al., 1991; Vrtala et al., 1993a). The epitope clones were then characterized by hybridization with 3 synthetic oligonucleotides spanning the Phl p I cDNA (Oligo A: 5'GGG GGC TTG TCC ACA TCC TTG TAC CCG C3' bp 191-218, oligo B: 5'GGA GAG GTC GAA GTG GTA GGG G3' bp 372-393, Oligo C: 5'CCG CCA CCA CGT CTC CGT CGC CG3' bp 573-595). Nineteen clones that had hybridized with only one of the oligonucleotides

were tested for IgE-binding with sera from 12 Phl p I allergic patients. Clones 80, 97 and 98 bound IgE from most patients and were further tested for IgE-reactivity with sera from 90 grass pollen allergic patients as described (Valenta et al., 1992). For
5 DNA sequence analysis, phage DNA was prepared, the epitope encoding cDNAs were excised with KpnI/SacI, subcloned into plasmid pUC 18 and both DNA strands were sequenced using lambda gt11 forward and reversed primers (Clontech, Palo Alto, USA) with ³⁵S dCTP (Sanger et al., 1977).

10

Expression and purification of a immunodominant recombinant Phl p I epitope.

The epitope encoded by clone 98 which bound IgE from 40 % of 90 grass pollen allergic patients and contained the shortest cDNA
15 fragment was selected for purification. Recombinant clone 98 phage were used to infect lysogenic E. coli 1089 (Huynh et al., 1985). The Phl p I epitope encoded by clone 98 was expressed as a β -galactosidase fusion protein in liquid culture (Huynh et al., 1985) and was affinity purified using an anti- β -galactosidase
20 affinity column (Promega, Maddison, USA) as described (Vrtala et al., 1993a). β -galactosidase was obtained upon infection of E. coli Y1089 with empty lambda gt11 phage and purified in the same way.

25 IgE-binding of natural timothy grass pollen allergens and recombinant Phl p I epitopes.

Natural timothy grass pollen allergens were extracted from timothy grass pollen (Allergon, Valinge, Sweden) (Vrtala et al., 1993b) separated by SDS-PAGE (Laemmli et al., 1970) and
30 transferred to nitrocellulose (Towbin et al., 1979). The recombinant Phl p I epitope (clone 98) and β -galactosidase were purified and also blotted on nitrocellulose. IgE from grass pollen allergic patients was used to detect the nitrocellulose blotted proteins as described (Jarolim et al., 1989) whereas IgE-
35 binding to non-denatured recombinant epitopes was done using plaquelifts of phage clones as described elsewhere (Spitzauer et

al., 1993). IgE-binding synthetic peptides which were prepared by Cambridge Research Biochemicals, U.K. was measured by dot blot assays. One hundred nanograms to two mikrogramms peptide per spot were dotted to nitrocellulose (Schleicher & Schuell, Dassel, Germany). Reactive peptides derived from other allergens and reactive sera were included as positive controls.

In vitro histamine release from basophils of patients.

Four grass pollen allergic patients with strong IgE-reactivity to group I grass pollen allergens were selected according to case history, serological testing, using RAST and immunoblotting with natural and recombinant grass pollen allergens and skin-prick test as described (Valenta et al., 1992). After informed consent was obtained heparinized blood samples were taken and granulocytes were prepared by dextran sedimentation (Valenta et al., 1989). Granulocytes were then incubated with increasing doses of natural timothy grass pollen allergens, anti-IgE mAb E-124-2-8 (positive control), the recombinant β -galactosidase fused Phl p I (clone 98) epitope and β -galactosidase (negative control), respectively. Liberated histamine expressed as percentage of total histamine was measured in the cell free supernatants by radioimmunoassay (Immunotech, Marseille, France) (Valenta et al., 1989).

To ensure that IgE-antibodies specific for clone 98 were present in the serum of the patient when histamine release was performed, the supernatants which were obtained from the granulocyte preparation were probed in parallel with nitrocellulose blotted timothy grass pollen allergens and the Phl p I (clone 98) epitope as described (Valenta et al., 1993b). The supernatants were from four grass pollen allergic individuals and from a non-allergic control individual. A buffer control without addition of a supernatant was also run.

RESULTS

Isolation and characterization of an immunodominant IgE-epitope clone from Phl p I.

100,000 phages from the Phl p I epitope library were screened using serum IgE from a grass pollen allergic individual with IgE-
5 reactivity to group I grass pollen allergens. 114 IgE-binding phage clones were obtained and subsequently tested for hybridization with 3 synthetic oligonucleotides spanning the Phl p I cDNA. 19 clones which hybridized with only one oligonucleotide were further tested with serum IgE from 12
10 different Phl p I allergic patients. All tested patients displayed IgE-reactivity with clone 98 which therefore contained an immunodominant epitope. Clone 80 reacted with ten out of twelve Phl p I reactive patients and clone 97 with eleven out of twelve patients. When tested with serum IgE from 90 grass-pollen
15 allergic patients which were selected according to case history, RAST (radioallergosorbent test) and skin prick tests, 40 % of the tested sera showed IgE-reactivity with clone 98 whereas 35 % reacted with clone 80 and clone 97 (data not shown).

The cDNAs of the three immunodominant epitope clones were
20 sequenced and found to code for almost the same portion of the Phl p I molecule. Figure 2 shows the alignment of the deduced amino acid sequences of the epitope clones with the amino acid sequences of the major grass pollen allergen from timothy grass Phl p I (Laffer et al., 1993). The amino acid of clone 98 was
25 aligned with the amino acid sequences of the other major grass pollen allergens from rye grass (*Lolium perenne*), Lol p I (Perez et al., 1990; Griffith et al., 1991), and from rye from timothy grass (*Phleum pratense*), Sec c I (Laffer et al., unpublished data). It was found that Clone 98 which reacted with IgE from all
30 patients contained a 15 amino acid immunodominant IgE-epitope of Phl p I which is highly conserved among the other group I allergens and covers amino acid 101-115 of the mature Phl p I protein. The C-terminal portion of the peptide seemed to be
critical for IgE-binding because clone 80 and 97 were less
35 frequently recognized. Peptides of 12 amino acids length were synthesized according to the deduced amino acid sequence of Phl p

I with 3 amino acids overlaps, spanning the complete allergen. No reactivity of the patients' IgE with these peptides could be detected indicating that the critical length for IgE-binding of the clone 98 epitope is in the range between 13-15 amino acids (data not shown). Two sythetic peptides (D: aa 76-87, E: aa 117-129) which flanked the clone 98 IgE-epitope also did not bind IgE (data not shown).

10 Comparison of different methods for the determination of B-cell epitopes.

Different methods were used to determine IgE-epitopes (= B-cell epitopes) of Phl p I. Overlapping peptides (Geysen et al., 1987) with a length of 12 amino acids which were synthesized according to the deduced amino acid sequence of Phl p I were tested by dot blotting for reactivity with serum IgE from grass pollen allergic patients. In addition ten peptides covering regions of a predicted high antigenicity (Jameson et al., 1988) of the mature Phl p I allergen:

- peptide A: aa 1-17
- 20 peptide B: aa 20-39
- peptide C: aa 44-69
- peptide D: aa 76-87
- peptide E: aa 117-129
- peptide F: aa 136-147
- 25 peptide G: aa 151-159
- peptide H: aa 177-193
- peptide I: aa 199-209
- peptide J: aa 214-237)

were also tested for IgE-reactivity with 35 sera from grass pollen allergic patients by dot blotting. None of the peptides tested reacted with IgE from more than 5 % of the grass pollen allergic patients (data not shown).

Since the epitope mapping based on the use of synthetic peptides was unsuccessful, a recombinant mapping strategy was used (Mehra et al., 1986). An epitope expression cDNA library was constructed using randomly fragmented Phl p I cDNA. 114 IgE-

epitope clones could be isolated out of 100,000 phages which were screened with IgE from sera of patients. 19 epitope clones containing cDNAs that hybridized only with one oligonucleotide and, therefore, contained small Phl p I fragments were selected. 5 These clones were further tested with sera from 12 different grass pollen allergic patients to determine immunodominant IgE-epitopes. One prominent IgE-epitope encoded by clone 98 was isolated. This sequence was not predicted to be antigenic by computer analysis (Jameson et al., 1988) nor was it identified by 10 testing overlapping synthetic peptides spanning the complete Phl p I molecule (Geysen et al., 1987).

IgE-binding capacity of recombinant Phl p I epitopes.

Recombinant Phl p I epitopes were expressed as β -galactosidase 15 fusion proteins and tested for IgE-binding as native and denatured proteins. β -galactosidase produced by lambda gt11 phage without insert was used as a negative control. Native recombinant IgE-epitopes were obtained by overlaying plates of recombinant E. coli/phage with IPTG soaked membranes (Valenta et al., 1992) 20 whereas for the assays under denaturing conditions, IgE-epitopes were purified by affinity to anti- β -galactosidase antibodies and were separated by denaturing SDS-PAGE (Laemmli et al., 1970) and electroblotted to nitrocellulose (Towbin et al., 1979). Clone 98 bound IgE from all 12 Phl p I allergic patients and when tested 25 with sera from 90 grass pollen allergic patients which were selected according to clinical criteria (case history, RAST and skin prick test) 40 % were found reactive.

Although clone 98 represented the immunodominant epitope some clones seemed to possess a higher IgE binding capacity. The 30 denatured immunoblotted clone 98 epitope also bound IgE from group I allergic patients. Compared with natural timothy grass pollen extracts a different intensity of IgE-binding was observed. This may be explained by the presence of group V allergens which comigrate with group I allergens in natural 35 extracts. β -galactosidase did not bind IgE in any of the two assays.

Identification of the immunodominant recombinant Phl p I IgE-epitope (clone 98) as a IgE-hapten by in vitro histamine release assays.

5 The recombinant Phl p I IgE-epitope encoded by clone 98 was tested for its capacity to release histamine from basophils of grass pollen allergic patients. Although all of the three patients displayed distinct IgE-reactivity to the nitrocellulose blotted recombinant Phl p I epitope, no histamine release was
10 observed when the patients granulocytes were incubated with the purified epitope. A dose dependent and specific histamine release was measured when granulocytes from patients were incubated with natural grass pollen allergens and anti-IgE mAb (positive control) whereas no release was obtained upon incubation with β -
15 galactosidase (negative control). One grass pollen allergic patient 98 epitope but with high levels of IgE against group V allergens was also included in the histamine release assays. This patient showed no histamine release with the clone 98 epitope whereas a dose dependent histamine release could be obtained with
20 total grass pollen extracts that contained group V allergens.

DISCUSSION

The present study demonstrates the efficient determination of IgE-epitopes using recombinant techniques. The cDNA coding for
25 the major grass pollen allergen, Phl p I (Valenta et al., 1992; Laffer et al., 1993), which is the target for IgE-antibodies of 90 % of the grass pollen allergic patients, and thus is the target for IgE-antibodies of up to 75 % of all allergic patients (Freidhoff et al., 1986; Valenta et al., 1992) was selected as
30 starting material to construct an epitope expression cDNA library. Using IgE of grass pollen allergic patients, an immunodominant IgE-epitope clone containing a Phl p I peptide having a length of 15 amino acids was isolated.

The described 15 amino acids epitope was not predicted by a
35 computer algorithm (Jameson et al., 1988) nor was it detected by overlapping peptide synthesis technology (Geysen et al., 1987).

The knowledge of IgE-epitopes is of particular importance because release of biological mediators such as histamine during the allergic effector reaction requires a divalent cross-link by allergens of IgE bound to mast cells and basophils (Segal et al., 5 1977). IgE-haptens derived from allergens contain only one IgE-epitope and thus cannot trigger allergic effector mechanisms unless they are polymerized. The immunodominant IgE-epitope which we derived from the major grass pollen allergen Phl p I was therefore purified and tested for its capacity to induce 10 histamine release from the basophils of grass pollen allergic patients. Although in all experiments histamine release could be induced with natural timothy grass pollen allergens in a dose dependent way, no histamine release could be elicited with the recombinant Ph p I epitope which therefore represents an 15 immunodominant IgE-hapten.

IgE-haptens may be useful for two therapeutical approaches of allergic disease: Haptens could be used to block mast cell- and basophil-bound IgE thereby directly inhibiting mediator release. Synthetic epitopes could be synthesized in large amounts and used 20 directly in the effector organs (lung nasal mucosa, conjunctiva). Such an approach would however require the characterization of many different haptens according to the IgE-binding pattern of the patient. Using recombinant DNA techniques for the characterization of allergens and the determination of IgE- 25 epitopes such an approach may be feasible. In addition, it is expected that due to extensive immunological crossreactivities among most allergens it might be possible to define a limited number of IgE-epitopes (Valenta et al., 1993a). As was demonstrated for Phl p I, IgE-epitopes can be determined by 30 immunoscreening of expression libraries derived from cDNAs of allergens. A representative number of patients can then be tested for IgE-reactivity with recombinant epitope clones to obtain immunodominant structures. In a second step most of the additional relevant IgE-epitopes of each allergen have to be 35 characterized. This might be possible because B-cell epitopes, unlike T-cell epitopes, assemble a larger conformation that also

has to be available on the surface of the allergen (Berzofsky et al., 1985; Chothia et al., 1991; Laver et al., 1990). thus it can be assumed that the diversity of B-cell epitopes may be much more restricted than that of T-cell epitopes.

5 In addition to the blocking of the allergic effector reaction, IgE-haptens could also be used to modulate IgE-responses by applying vaccination strategies which aim at the induction of immunological tolerance. Hyposensitization treatment of Type I allergies which is established world wide requires the increasing
10 application of allergens by injection or oral administration. Although hyposensitizations is used successfully since 1911 (Noon et al., 1911), many patients undergoing this therapy suffer from severe side effects such as anaphylactic shock. The use of IgE-haptens derived from the allergens might contribute considerably
15 to the improvement of this therapy by reduction of such side effects. Different other methods to modulate the IgE-response in allergic patients which are currently developed might also take advantage from the use of IgE-haptens.

In conclusion our study demonstrates that by use of recombinant
20 techniques an immunodominant IgE-hapten from the major timothy grass pollen allergen Phl p I could be obtained. This allergen was selected as a model allergen because a high proportion (> 90 %) of all grass pollen allergic patients, and thus almost 75 % of all allergic patients show IgE-reactivity with this molecule.
25 Although the obtained 10 amino acids epitope bound IgE from approximately 40 % of grass pollen allergic patients, it did not release histamine from the basophils of patients and may therefore be considered as a hapten. We are aware that in addition to the described Phl p I epitope certainly more epitopes
30 from the same molecule and other allergens must be defined to block histamine release by saturating mast cells and basophils of allergic patients. However, the results encourage to continue with the characterization of IgE-haptens for their future use in specific therapy of allergic diseases.

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SEQUENCES 1. cDNA and deduced amino acid sequence of Phl p I epitope clones discovered up to the priority date (c80, c97, c98).

c80: CAC ATC ACC GAC GAC AAC GAG GAG CCC ATC GCC CCC TAC CAC TTC

5 H I T D D N E E P I A P Y H F

GAC CTC TCC GGC CAC GCG

D L S G H A

10 C97: AAC GAG GAG CCC ATC GCC CCC TAC CAC TTC GAC CTC TCC GGC CAC

N E E P I A P Y H F D L S G H

GCG TTC GGG

A F G

15

C98: GCC CCC TAC CAC TTC GAC CTC TCC GGC CAC GCG

A P Y H F D L S G H A

SEQUENCES 2. Alignment of the deduced amino acid sequences of all
20 Phl p I IgE-epitope clones with the full amino acid sequence of
the Phl p I allergen.

The clone number is at the right end. The full amino acid
sequence of the Phl p I allergen is given by the longest lines.
The underlined part corresponds to the signal peptide and is not
25 present in the mature polypeptide.

MASSSSVLLVYVLEAYELGSAYGIPKVP PPGPNITATYGD KWLDAKSTWY GKPPTG
IPKVP PPGPNITATYGD KWLDAKSTWY GKPPTG

AGPKDNGGACG YKDV DKKPPPFSGM TGC GNTPIFKS GRGCGSCFEIKCTKPEACSG
AGPKDNGGACG YKDV DKKPPPFSGM TGC GNTPIFKS GRGCGSCFEIKCTKPEACSG 113
GYKDV DKKPPPFSGM TGC GNT 45

EPV VVHI TDDN EEPIA PYHFDLSG HAFGAMAKKGDEQK LRSAGELELEQFRRVKC
PV VVHI TDDN EEPIA PYHFDLSG HAFGAMAKKGDEQK LRSAGELELEQFRRVKC 43
HI TDDN EEPIA PYHFDLSG HAFGAMAKKGDEQK LRSAGELELEQFRRVKC 34
HI TDDN EEPIA PYHFDLSG HAFGAMAKKGDEQK LRSAGELELEQFRRVKC 80
TDDN EEPIA PYHFDLSG HAFGAMAKKGDEQK LRSAGELELEQFRRVKC 114
TDDN EEPIA PYHFDLSG HAFGAMAKKGDEQK LRSAGELELEQFRRVKC 95
TDDN EEPIA PYHFDLSG HAFGAMAKKGDEQK LRSAGELELEQFRRVKC 50
TDDN EEPIA PYHFDLSG HAFGAMAKKGDEQK LRSAGELELEQFRRVKC 97
TDDN EEPIA PYHFDLSG HAFGAMAKKGDEQK LRSAGELELEQFRRVKC 103
TDDN EEPIA PYHFDLSG HAFGAMAKKGDEQK LRSAGELELEQFRRVKC 98

KYPEG TKVTFHVEKGSNPNYLLV.KYVNGDGDVVAVDIKEKGKDKWIELKESW
HVEKGSNPNYLLV.KYVNGDGDVVAV 64

GAIWRIDTPDKLTGPPFTVRRYTTTEGGGTTKTEAEEDVVIPEGWKADTSYESK 109
LTGPPFTVRRYTTTEGGGTTKTEAEEDVVIPEGWKADTSYESK 86
GPPFTVRRYTTTEGGGTTKTEAEEDVVIPEGWKADTSYESK 41
RYYTTTEGGGTTKTEAEEDVVIPEGWKADTSYESK 108
RYYTTTEGGGTTKTEAEEDVVIPEGWKADTSYESK 42
RYYTTTEGGGTTKTEAEEDVVIPEGWKADTSYESK 52

P A T E N T C L A I M S

1. A recombinant DNA molecule comprising a nucleotide sequence (I) which codes for a polypeptide displaying the antigenicity of one, two or more of the Phl p I epitope clones 28, 34, 41, 42, 43, 50, 52, 64, 80, 85, 86, 95, 97, 98, 103, 108, 109, 113, 114 with the amino acid sequences defined in figure 2 and preferably being derived from grasses or monocotyledonic plants, or a nucleotide sequence (II) which hybridizes with such a nucleotide sequence (I) under conditions of high stringency.
2. A recombinant DNA molecule according to any one of claims 1-2, comprising a nucleotide sequence which codes for a polypeptide having antigenic crossreactivity and a high degree of homology, preferably > 50 % such as > 60 % or > 75 %, with Phl p I epitopes from grasses or other monocotyledonic plants, preferably those defined by the amino acid sequences given in figure 2 for clones 28, 34, 41, 42, 43, 50, 52, 64, 80, 85, 86, 95, 97, 98, 103, 108, 109, 113, 114.
3. A recombinant DNA expression vector or cloning system comprising an expression control sequence operatively linked to a recombinant molecule defined in any one of claims 1-2.
4. A recombinant expression vector in which an expression control sequence is operationally linked to a coding nucleotide sequence which hybridizes with a Phl p I cDNA clone defined by the amino acids sequences given in figure 2, said hybridization taking place under conditions of high stringency.
5. A host cell containing a recombinant molecule or vector according to any one of claims 1-4.
6. A polypeptide displaying the antigenicity of at least one of the Phl p I epitopes defined by the amino acid sequences given in clones 28, 34, 41, 42, 43, 50, 52, 64, 80, 85, 86, 95, 97, 98,

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103, 108, 109, 113, 114 of figure 2 or a Phl p I unique portion of these sequences.

7. A polypeptide according to claim 6 in which the synthetic protein or polypeptide is fused to an additional polypeptide.

8. A polypeptide according to claim 7 wherein said additional polypeptide is β -galactosidase, GST or lambda cII protein or any other polypeptide that can be expressed as a fusion protein in prokaryotic or eukaryotic cells.

9. Method of diagnosing allergy, preferably in vitro, to plant proteins in a patient which comprises contacting a body fluid sample, suspected of containing antibody against the plant protein, with a polypeptide defined in claims 6-8 under conditions permitting the formation of a complex containing the antibody and the polypeptide, whereafter the complex is measured and related to the amount of the antibody in the sample, an elevated level being taken as an indication of allergy against a plant protein comprising the polypeptide.

10. Method for measuring, preferably in vitro, the cellular reaction against a Phl p I epitope, in which a recombinant or synthetic protein or polypeptide according to any of claims 6-8 is used to stimulate the cellular reaction.

11. Method of treatment of an animal which has a pollen allergy by administering a therapeutically effective amount of a recombinant or synthetic protein or polypeptide according to any of claims 6-8.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 95/00724

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/415, C12N 15/29, A61K 39/36, C07K 14/735
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REG, CA, BIOSIS, WPI, CLAIMS, GENESEQ SWISSPROT, EMBL, PIRONLY

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 9421675 A2 (IMMULOGIC PHARMACEUTICAL CORPORATION), 29 Sept 1994 (29.09.94)	1-8
X	WO 9203550 A1 (THE UNIVERSITY OF MELBOURNE), 5 March 1992 (05.03.92)	1-8
X	WO 9410314 A1 (THE UNIVERSITY OF MELBOURNE), 11 May 1994 (11.05.94)	1-8
X	THE JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 265, No 27, Sept 1990, Mary Perez et al, "cDNA Cloning and Immunological Characterization of the Rye Grass Allergen Lol p I", page 16210 - page 16215	1-8

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

6 October 1995

Date of mailing of the international search report

11-10-1995

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE95/00724

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 9-11
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

28/08/95

International application No.

PCT/SE 95/00724

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A2- 9421675	29/09/94	NONE	
WO-A1- 9203550	05/03/92	AU-B- 659801	01/06/95
		AU-A- 8408391	17/03/92
		CA-A- 2089735	18/02/92
		EP-A,A- 0576426	05/01/94
		JP-T- 5509230	22/12/93
WO-A1- 9410314	11/05/94	NONE	

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